

THE EFFECT OF COFFEE SUPPLEMENTATION ON GLUTATHIONE AND TOTAL THIOLS LEVELS

ZANETA BUCHTOVA¹, ZUZANA LACKOVA^{1,2}, JIRI KUDR^{1,2},
VOJTECH ADAM^{1,2}, ONDREJ ZITKA^{1,2}

¹Department of Chemistry and Biochemistry
Mendel University in Brno
Zemedelska 1, 613 00 Brno

²Central European Institute of Technology
Brno University of Technology
Technicka 10, 616 00 Brno
CZECH REPUBLIC

zaneta.buchtova@mendelu.cz

Abstract: Antioxidants are very important substances that counteract the formation of free radicals. They are divided into exogenous, such as vitamin C which the body receives with food, and endogenous. One of the most important endogenous antioxidants is glutathione (γ -glutamyl-L-cysteinyl-glycine), which plays an important role in cellular defense against oxidative damage. Free glutathione is presented within organisms in both reduced (GSH) and oxidized forms (GSSG). Oxidative stress leads to a decrease in GSH level and therefore a GSH/GSSG ratio that can be used as an indicator of oxidative stress and an indicator of various diseases. The aim of presented study was to develop a sampling method for capillary blood testing, where we have found that this amount (15 μ l) is sufficient for our testing. Another aim was to supplement group of volunteers with coffee and to determine GSH levels and levels of total thiols after 0, 48 h and 96 h of supplementation in capillary blood. HPLC with electrochemical detection was used for GSH determination and Ellman's method for determination of total thiols. We could see the GSH slight increase as well as the levels of total thiols.

Key Words: antioxidant, coffee, glutathione, HPLC-ED, total thiols

INTRODUCTION

Antioxidants are extremely important molecules preventing negative effects of free radicals. Oxidative stress results in damage to DNA, proteins, lipids and carbohydrates and is the cause of many human diseases (Carru et al. 2003, Childs et al. 2016, Squellerio et al. 2012). Glutathione is one of the most important intracellular non-enzymatic antioxidant (Giustarini et al. 2011, Wu et al. 2004). GSH contains a thiol group in its molecule that is very reactive. Thiols have been and continue to be of interest because they play an important role in a number of biological processes (Giustarini et al. 2015, Rossi et al. 2006). The primary function of glutathione is to remove reactive oxygen species (ROS) (Kominkova et al. 2015, Minelli and Gogele 2011, Zhang et al. 2014). It also has many other important physiological functions (Childs et al. 2016, Townsend et al. 2003, Wu et al. 2004). The experiment was conducted on a beverage with proven antioxidant effects – coffee. Coffee consists of several biological active compounds, such as caffeine, diterpenes, chlorogenic acids, and melanoidins, which may affect human health (Godos et al. 2014). The most important antioxidants in coffee are polyphenols and the most represented polyphenol with antioxidant effects is chlorogenic acid. Studies in recent years have generated new information regarding the effects of coffee consumption on health, disproving the common belief that coffee is mostly harmful. A number of recent experimental and epidemiological studies reported a substantial positive effect of coffee consumption on human health, especially in relation with cardio-metabolic risk factors (Abraham et al. 2013, Bakuradze et al. 2011, Ludwig et al. 2014, Salomone et al. 2014). Data published by Jung et al. suggest that coffee has a physiological antioxidative and anti-inflammatory effect and these effects are negatively correlated with roasting levels where antioxidant activity decreases with roasting time (Jung et al. 2017, Kotyczka et al. 2011).

For such experiments venous blood is commonly used (Wink et al. 2016), but for the detection of glutathione in the blood by HPLC-ED and for spectrophotometric assay we can use a small amount of capillary blood. This study is focused on supplementing a selected sample of coffee according to the highest amount of polyphenols and determining GSH and total thiols in capillary blood.

MATERIAL AND METHODS

Chemicals

GSH and GSSG, 5,5'-dithiobis-(2-nitrobenzoic acid), cysteine, sodium acetate, Coomassie Brilliant Blue G-250, Folin-Ciocalteu reagent (FCR), phosphoric acid, ferulic acid and trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich (St. Louis, MA, USA). Methanol in HPLC grade was obtained from Chromservis (Prague, Czech Republic).

Biological material

The capillary blood was collected using the capillary from the heated (60 °C) lateral part of the finger. A single lancet pen was used for injection. In total, 10-15 µl of blood was collected and divided into three aliquots. All volunteers provided informed agreement and the experiment was approved by the ethics committee of Mendel University in Brno.

Determination of total polyphenols

Folin-Ciocalteu method was used to determine total polyphenol compounds. Initial calibration was performed on ferulic acid. 250 µl of FCR and 10 µl of sample were pipetted into the tubes, then all the tubes were mixed. Then 200 µl of 7.5% sodium carbonate solution was added. Samples were incubated at room temperature for 30 min. The colored product of reaction was determined using Infinite M200Pro (Tecan, Männedorf, Switzerland) at 580 nm.

HPLC-ED

Analysis of GSH and GSSG was performed using HPLC-ED comprising two chromatographic pumps, twelve-channel CoulArray electrochemical detector column containing reverse phase Zorbax eclipse AAA C18. Detector consisted of three flow analytical chambers (Zitka et al. 2011). Mobile phase consisted of A: TFA-water (3:97, w/w) and B: 100% methanol. The most optimal preparation procedure found from optimization: an aliquot of 5 µl was taken from the collected blood (15 µl) and diluted with 45 µl of 10% TFA, followed by freezing in liquid nitrogen for approximately one minute. Each sample was then sonicated with an ultrasonic needle for 30 seconds, vortexed and centrifuged for 20 minutes at 25,000 rpm and 4 °C. Approximately 40 µl were taken from each sample.

Total thiols analysis

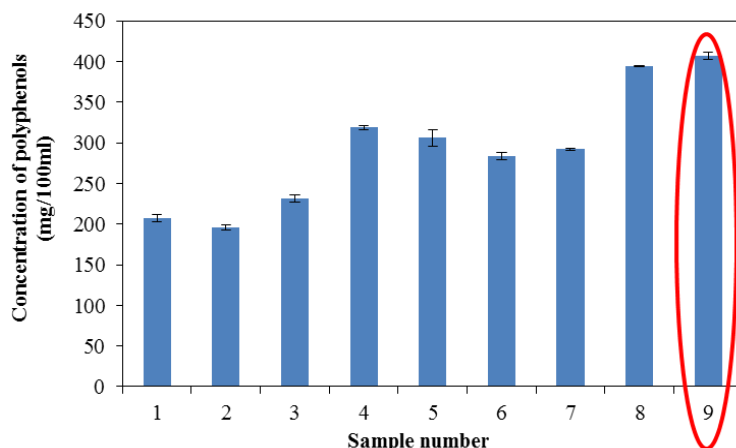
Blood samples (5 µl, five times diluted with water) were mixed with 138 µl of Ellman's reagent (2 mM 5,5'-dithiobis-(2-nitrobenzoic acid) in 50 mM sodium acetate). The reaction was started using addition of 16.5 µl Tris base buffer (1 M, pH 8 was adjusted using acetic acid). The colored product of reaction (159.5 µl) was determined using Infinite M200Pro at 436 nm within 96-well plate with flat bottom.

Bradford's assay

Bovine serum albumin was used as a standard. Bradford's reagent was prepared as follows: 10 mg of Coomassie Brilliant Blue G-250 was dissolved in 5 ml of 100% ethanol, subsequently 10 ml of 85% phosphoric acid was added and solution was filled to 100 ml with distilled water. 90 µl of Bradford's reagent was mixed with blood samples. The coloured product of reaction was determined using Infinite M200Pro at 595 nm. The scheme is shown in Figure 1.

RESULTS AND DISCUSSION

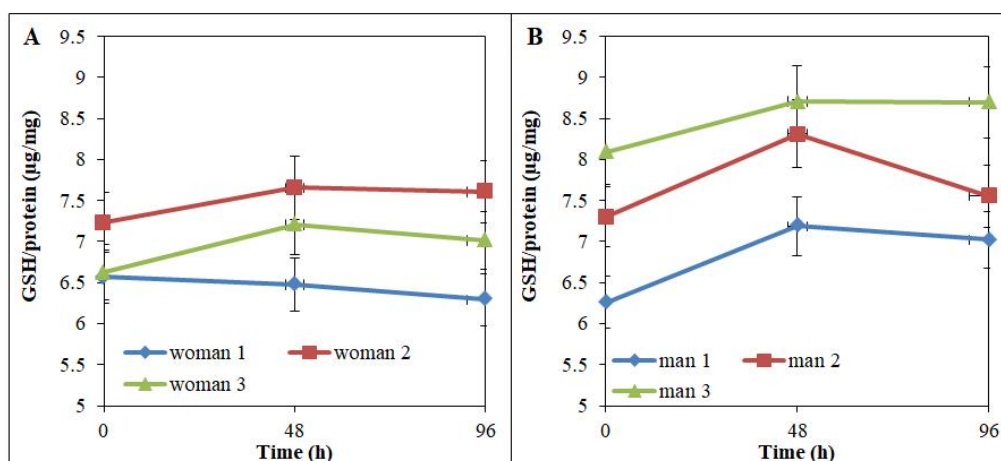
The measured data is an average of three determinations. First, the total amount of polyphenols was determined in 9 samples of coffee using Folin-Ciocalteu method. 2.5 g of coffee was poured into 100 ml of hot water (80 °C) and filtered after 5 minutes.

Figure 1 Concentration of polyphenols in 9 samples of roasted coffee.

For the experiment with the supplementation with roasted coffee, a sample 9, which contained the highest concentration of polyphenols (namely 407.1 mg/100 ml of coffee \pm 4.81), was selected (Figure 1). The lowest concentration of polyphenols was detected in sample 2.

Content of GSH

Six volunteers took part in the experiment (23–30 years). Optimized method for GSH/GSSG analysis was used for investigation of GSH levels in capillary blood samples of group of six volunteers supplemented with coffee. Their GSH levels were determined before the start of supplementation, 48 and 96 h after supplementation. The dose of coffee was calculated according to volunteer weight (2.5 g of coffee per 50 kg of weight for 1 cup) and 4 cups of coffee per day. Limited intake of food and beverages with high antioxidant concentrations like green tea, wine, fruit, vegetable and dietary supplements was recommended to volunteers. 15 μ l of capillary blood were taken and GSH levels and total protein concentrations were determined.

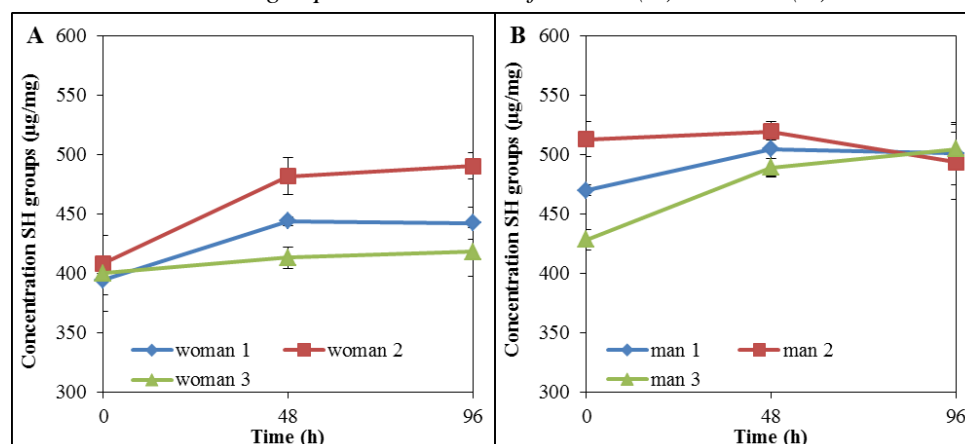
Figure 2 GSH levels during experiment in case of women (A) and men (B).

From the results, we can conclude that consumption of coffee induced a slight increase of GSH concentration in the blood (Figure 2). No increase was observed in case of women, but some changes were observed in case of men. Increases occurred mainly between first and second sampling. At the third sampling, the concentration was mostly stagnant or slightly declining. This may be due to the fact that high antioxidant intake resulted in GSH pool saturation. GSH levels elevation after coffee consumption also reported (Bakuradze et al. 2011).

Another results suggested that coffee didn't induced statistically significant changes of GSH levels during the test period (Teekachunhatean et al. 2012). Another important outcome is that we are able to perform this analysis even from a small amount of capillary blood and which causes only a minimal discomfort for volunteers.

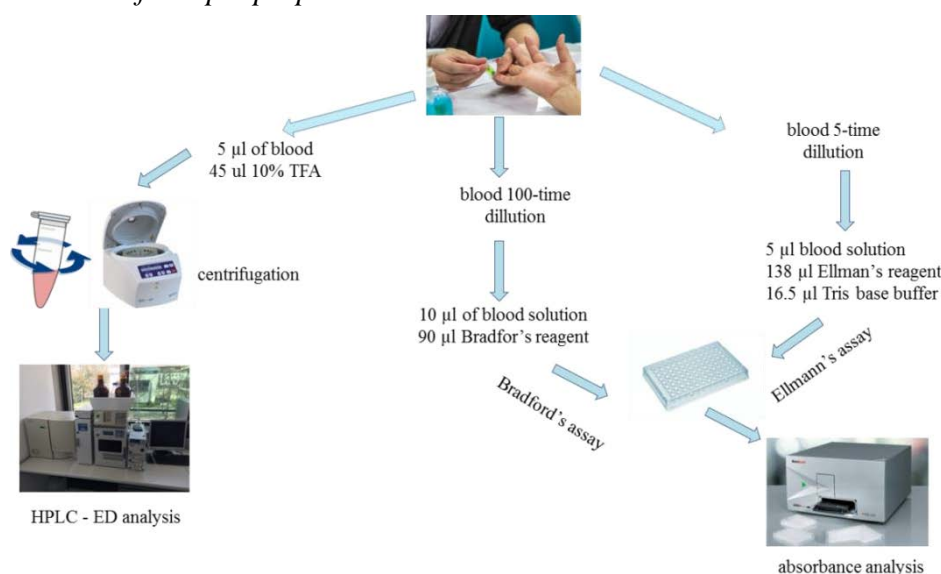
Content of total thiols

Figure 3 Total thiol levels during experiment in case of women (A) and men (B).



Analysis of total thiols was performed on a spectrophotometer using the Ellman's method. For this analysis, 2.5 µl of blood was needed, which was taken at the same time as blood for GSH determination and which was diluted with an appropriate amount of phosphate buffer. The results of determination of SH groups mostly correlate with GSH content. This also confirms the accuracy of the experimental data because GSH is one of the most abundant molecules in blood together with the SH group (Giustarini et al. 2016, Wang et al. 2014). Almost all volunteers who consumed coffee exhibited an increase in SH group concentrations in the first 48 hours. In the following days, this growth of total SH group concentrations was stabilized and even decreased (Figure 3). The scheme of sample preparation is shown in Figure 4.

Figure 4 Scheme of sample preparation



CONCLUSION

The aim of the study was to find out if we are able to observe changes in the content of thiol substances in capillary blood affected by supplementation with beverage containing high antioxidant concentration. First, it was important to optimize GSH assay methods on a high-pressure liquid chromatograph with electrochemical detection. The coffee experiment, which was attended by 6 volunteers (three men and three women), showed a slight increase in both GSH and total SH group levels.

The highest increase was observed between the first (the average value is 7.0 ± 0.6 µg GSH/mg protein) and the second sampling (the average value is 7.6 ± 0.7 µg GSH/mg protein). At the third

sampling, the increase was much smaller or even stagnated (the average value is $7.4 \pm 0.8 \mu\text{g GSH/mg protein}$). We showed that capillary blood is suitable for GSH/GSSG electrochemical analysis in fast short-term study where due to this method change of GSH levels can be determined prior to the change of the state of organism.

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